Effect of local air pollution on the sporophore production of mycorrhizal fungi, mycorrhizae and microbial activity in Scots pine forests

Effekten av lokal luftförorening på sporproduktionen hos mykorrhiza-svamp, mykorrhiza og mikrobiell aktivitet i tallskog

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Abstract

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The biology of forest soil humus layer was studied around an industrial city affected by a varied spectrum of air pollution. The work began in 1987 as part of the Finnish Research Project on Acidification (HAPRO) and is particularly concerned with the mycorrhizal and decomposing fungi in forest. The sites are dryish Scots pine forest stands in the environs of Oulu, northern Ostrobothnia (N 65°, E 25°30').

The species composition of the mycorrhizal fungi was different in the differently polluted areas. The biomass and number of sporophores and biological activity in the humus seemed to decrease towards the most polluted areas. Reasons for this may lie in the pH, the amount of ammonium nitrogen present, total nitrogen and total sulphur, all of which were at a higher level in the most polluted areas than in the cleanest areas. Sporophore production and biological activity showed negative correlations with these soil parameters. The mycorrhizae were in the poorest condition in the most polluted area.

Key words: Air pollution, fungi, mycorrhizae, biological activity, pine.

Sammandrag

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Biologin av skogsmarkens humuslager undersöktes i en industristads omgivningar som påverkas av flera olika luftföroreningar. Undersökningen påbörjades 1987 och utgör en del av det finländska undersökningsprojektet i försurning (HAPRO). Arbetet koncentrerar sig särskilt på undersökning av mykorrhiza- och rötsvampar in skogens

humuslager. Försöksytorna är tallskogsmarker i Uleåborgs omgivningar i Nordösterbotten (N 65°, E 25°30').

Mykorrhizasvampens artsammansättning varierade alltefter områdets föroreningsgrad. Sporangiernas biomassa och antal samt humusens biologiska aktivitet verkade avta mot de mest förorenade försöksytorna. Orsaken till detta kan vara pH, mängden av ammoniumkväve eller den totala mängden kväve eller svavel in humusen som alla låg på en högre nivå i de mera förorenade ytorna jämfört med de renare områdena. Sporangiumsproduktionen och den biologiska aktiviteten korrelerade negativt med dessa markfaktorer. Mykorrhizorna var i sämsta skick på de mest förorenade försöksytorna.

Nyckelord: Luftförorening, svamp, mykorrhiza, biologisk aktivitet, tall.

Introduction

The biological processes taking place in the soil are of prime importance for the fertility of forest ecosystems and changes in them may have ecologically and economically far-reaching effects on forest productivity. Important biological processes in forest soil include the decomposition of organic material and the mediation of the nutrient uptake of trees by mycorrhizal fungi. The environment of the microorganisms taking part in these processes is mainly the humus layer of the soil, which may undergo quite drastic changes due to the deposition of airborne pollutants, includig changes in pH, nutrient balance, and amounts of heavy metals, and also the vegetation and rhizosphere.

Research into the effects of air pollution on forest soils in the environs of Oulu was begun in 1987 as a part of the Finnish Research Project on Acidification (HAPRO). The work is particularly concerned with the effects of air pollution on the sporophores of the mycorrhizal fungus species and mycorrhizal and decomposing fungi in forest humus, but some chemical parameters regarding the organic matter in the soil were determined as background data for assessing the environmental conditions of microorganisms. Some preliminary results are presented in this raport.

Sites and methods

Sites

The 20 sites studied all represent dryish Scots pine forest stands in the environs of Oulu, Northern Ostrobothnia (N65°, E 25°30') and are located in four pollution zones determined according to the sulphur content of the pine needles (OHTONEN et al. 1989). Zone I is the cleanest and lies about 20 km from the emision sources, while zone IV comprises the central part of the city and is nearest to the emision sources. The precise locations of sites is presented in Fig. 1. The principal emisions in the area are of: SO_2 10–12 000, CO 10 000, NO_x 7–8 000, dust 7–8 000. H_2S 1 200, Pb 20 and Hg 0.2 tn/year (VUONONVIRTA et al. 1984).

The field layer of the vegetation at the sites composed mainly of *Vaccinium vitis-idaea, V. myrtillus* and *Empetrum nigrum* and the ground layer of *Pleurozium schreberi, Dicranum scoparium* and *D. polysetum.* The sites of the city centre have more herbs and grasses than the outermost sites, probably because of the effects of various pollutants, and it is difficult to determine the original forest types.

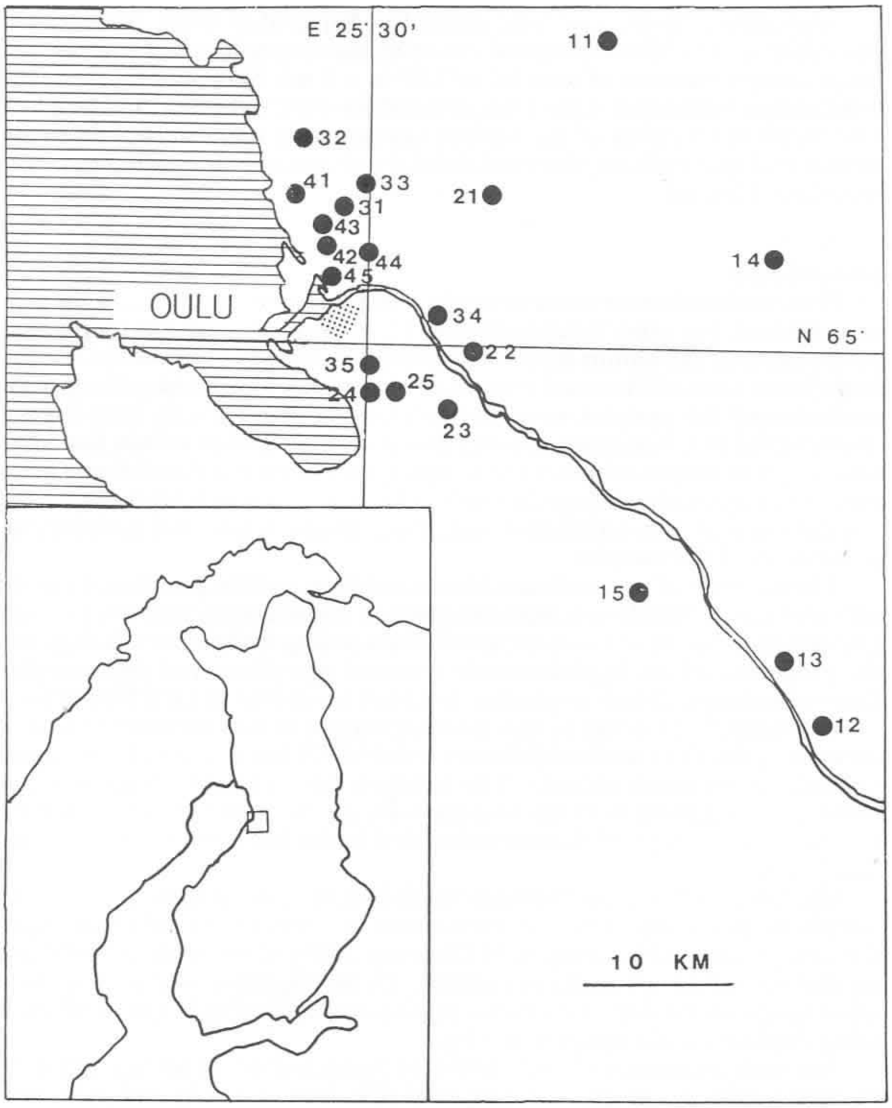


Fig. 1. The study area. Sites 11–15: zone I, sites 21–25: zone II, sites 31–35: zone III, sites 41–45: zone IV.

Sampling and methods

Sporophores

The species of mycorrhizal fungi present were assessed by making an inventory of the sporophores growing at the sites twice a month in August and September 1988. Preliminary inventories made in September 1987 were also used.

Sporophore production was studied in September 1988. Productivity was estimated by identifying and counting the sporophores of mycorrhizal fungi along a transect of area 50 m^2 (25 m x 2 m). Sporophores were also collected on September 12th-15th, dried at 60–70°C overnight and weighed. The mean dry weights of the various species were then multiplied by the numbers of sporophores observed at the sites to convert the earlier data into biomass estimates.

Soil analyses

Five random humus cores were taken in September for counting the pine mycorrhizae. For other determinations 15–35 cores at each site (depending on the depth of the humus layer) were taken five times from June to October. In the latter case, all litter and roots over 1 mm thick were removed before the analyses and the samples were homogenized by hand. Fresh material was analyzed for pH, biological activity and living mycelium within four days and the remaining portions of the samples were oven-dried and deep-frozen for later analysis of exchangeable nutrients, heavy metals, total nitrogen and sulphur content, loss on ignition, etc. These analyses have not yet been carried out on all the samples.

The amounts of mycorrhizae, their condition and the proportions of the different mycorrhizal types were determined under a dissection microscope. The mycorrhizae were roughly classified according to their morphology and the properties of the hyphal mantle, external mycelium and rhizomorphs. They were assigned to four condition classes according to LEHTO (1984).

The amount of living fungal mycelium present was measured twice in autumn by the FDA method (SÖDERSTRÖM 1977) from 3.0 g of fresh humus in triplicate or quadruplicate. The samples were studied microscopically using a Leitz Laborlux D microscope with an AG filter system and NPL fluotar 50 lens. The hyphae were calculated by the intersection method (OLSON 1950).

Microbial activity was measured both in terms of soil respiration and by dehydrogenase assay. The soil respriration rate was measured by the alkali absorption method (COLEMAN 1973) using 20.0 g of fresh sample and two incubations of 20–24 hours per sample. Dehydrogenase activity was measured by the method of THALMANN (1968) as modified by MALINEN (1983), using duplicate fresh samples of 2.5 g.

Ammonium and nitrate nitrogen were measured in 1 M KCl extract (1:10 by volume) by the colorimetric method (Black et al. 1965) and pH with a glass electrode in a soil-water extract (1:2 by volume) after shaking for 1 hour and allowing to stand for 0.5 hour. Soil moisture was measured gravimetri-

cally at 105°C, total nitrogen determined by the micro-Kjeldahl method with tube digestion (KUBIN 1978), and total sulphur determined by x-ray fluorescene analysis (OHTONEN et al. 1989).

Descriptive statistics (mean and standard error), ANOVA and correlation analysis were performed on some of the material.

Results and discussion

Species composition

The total number of mycorrhizal species producing sporophores in 1987 and 1988 (Fig. 2) was distinctly higher at the sites in the two cleaner zones, I and II (22.4 ± 2.9 and 19.6 ± 1.4, respectively), than in the more polluted zones III and IV (6.0 ± 1.9 and 6.0 ± 0.6, respectively). In most cases only 2–8 species were found in the two central zones, although sporophores of 13 mycorrhizal species were encountered in one stand (32). Twenty species or more were encountered at sites 11, 12 15, 21, 23 and 25. The lowest number of mycorrhizal fungus species, only two, was recorded at site 31.

Typical fungus symbionts of pine, such as *Suillus variegatus* and*Chroogomphus rutilus* were abundant in the two cleaner zones, but hardly occurred at all at the more polluted sites. Also *Cortinarius* spp. were less frequent in the more polluted zones, especially in zone III. In contrast *Paxillus involutus* occurred almost entirely in the two central zones with high nitrogen levels. *Lactarius rufus* was abundant at all the sites and increased slightly towards the most polluted areas. This species also seemed to produce sporophores later in the autumn at the more polluted sites than at the cleaner ones.

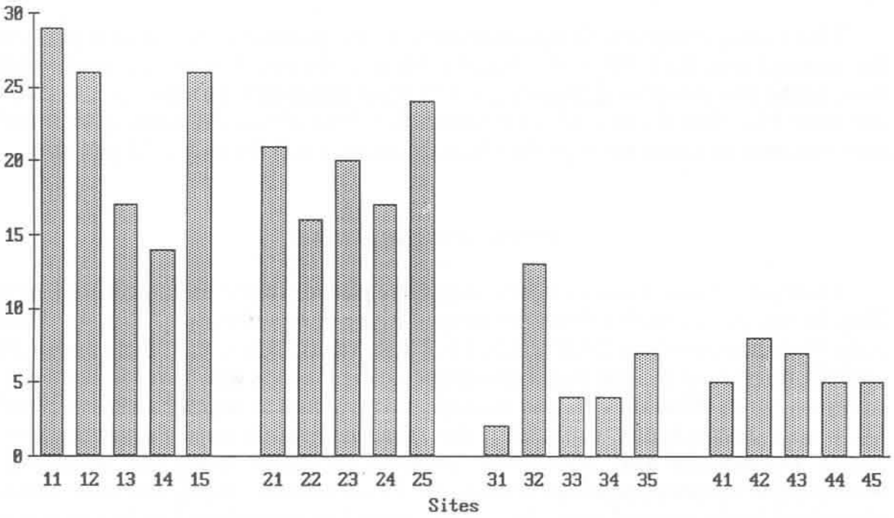


Fig. 2. Numbers of mycorrhizal species encountered at the sites in 1987 and 1988.

Biomass and density of sporophores

The biomass of sporophores of mycorrhizal fungi (Fig. 3) was distinctly higher in th cleaner zones I and II (1.19 ± 0.22 and 0.86 ± 0.30 g dw/m^2, respectively) than in zone III (0.25 ± 0.06 g dw/m^2), but was again higher (0.53 ± 0.23 g dw/m^2) at the central sites, where *Lactarius rufus* produced most of the sporophores.

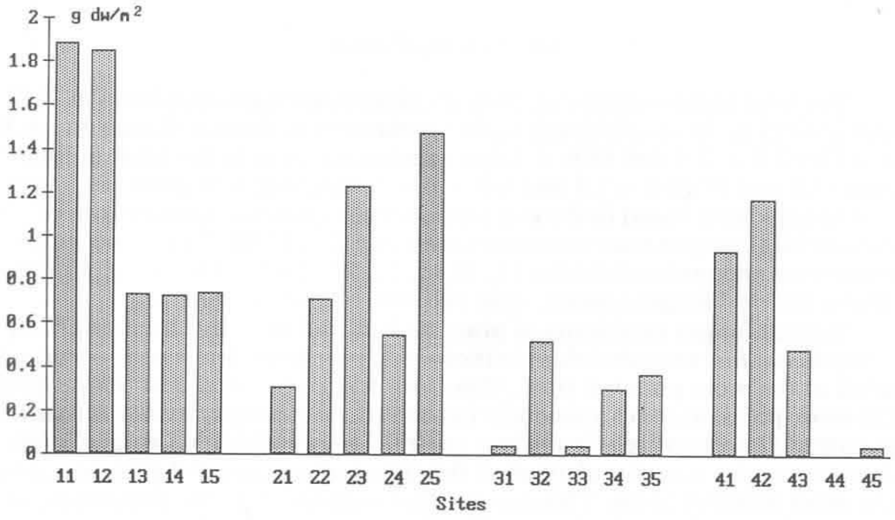


Fig. 3. The biomass of sporophores of mycorrhizal fungi in September 1988.

The average density of sporophores, as the biomass, too, was higher in the zones I and II (1.94 ± 0.43 and 1.54 ± 0.45 number/m^2, respectively) than in the more polluted area (0.24 ± 0.10 in the zone III and 0.55 ± 0.24 in the zone IV). Stands 41 and 42 nevertheless had about the same density of sporophores as some sites in the cleanest zone, i.e. 0.84 and 1.24 per m^2.

Ectomycorrhizae of pine

The ectomycorrhizae of Scots pine were poorer in the most polluted area (Fig. 4), the proportion of well developed mycorrhizae being 17.8 ± 4.7 % in zone IV, whereas it was 29.8 ± 3.2, 25.7 ± 4.3 and 28.6 ± 6.8 % in zones I, II and III, respectively. The number of mycorrhizal types was lower in the more polluted zones III and IV (6 and 9, respectively) than in zones I and II (12 and 14, resp.). Some types, especially the rhizomorphous ones *Piloderma croceum, Dermocybe* and *Hebeloma,* seemed to suffer from the pollution, whereas the ectomycorrhizae formed by *Cenococcum geophilum* were more abundant in the central area. This is discussed in more detail in MARKKOLA and OHTONEN (1988).

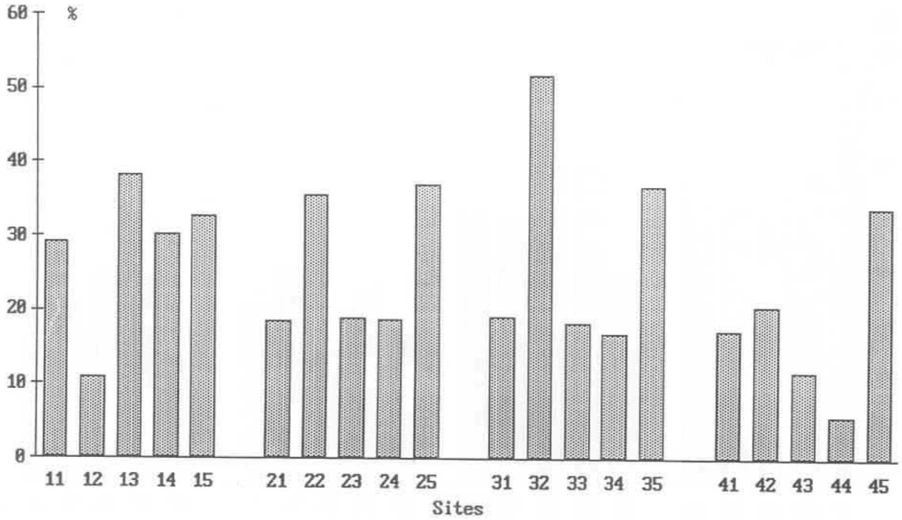


Fig. 4. Proportions of healthy ectomycorrhizae in Scots pine roots (% of all root tips) as means from five cores collected at each site in 1987.

Biological activity

Expressed in terms of the rate of humus respiration, biological activity was lower at the more polluted sites than at the cleaner ones (Fig. 5). The average respiration rates during the growing season were 51.9 ± 4.6, 45.2 ± 2.6, 31.2 ± 1.9 and 32.7 ± 4.8 µg CO_2/g/h in zones I, II, III and IV, respectively. The differences between the zones were highly significant ($p<0.001$).

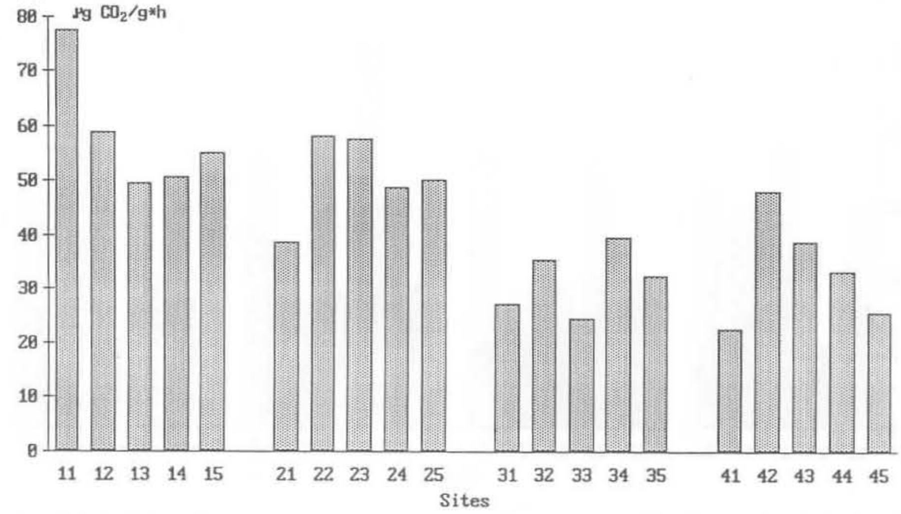
There were also significant differences in respiration rate between the sites in the most polluted zone (IV) ($p<0.01$), but not in the other zones. Site 41 had a markedly lower respiration rate than any other site.

The same trend applied to the dehydrogenase assay, activities being 1.43 ± 0.12, 1.27 ± 0.06, 1.10 ± 0.07 and 1.20 ± 0.12 µmol TPF/g/24 h in zones I, II, III, and IV, respectively. The zones did not differ significantly from each other. However, site 11 had a markedly higher dehydrogenase activity than any other site, and site 41 a markedly lower level.

Amount of living mycelium

The amount of living mycelium was measured twice, and only in zones I and IV. The average amount of the mycelium at the sites in zone IV was higher (610 ± 160 m/g) than zone I (320 ± 70 m/g), but the difference was not significant and was due to the very high amount measured in October at the site 42 (Fig. 6).

A



B

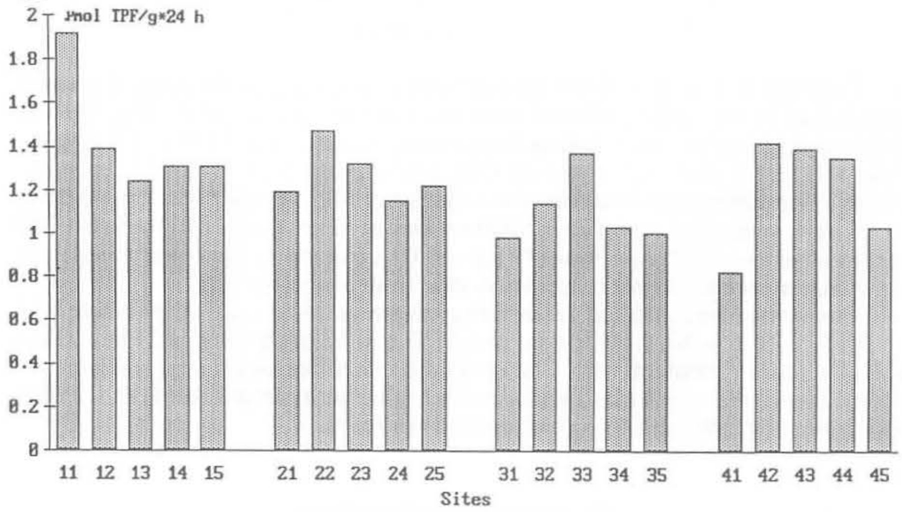


Fig. 5. Respiration rate (A) and dehydrogenase activity (B) in the humus layer in 1987 (means of 3 measurements for respiration and 5 for dehydrogenase).

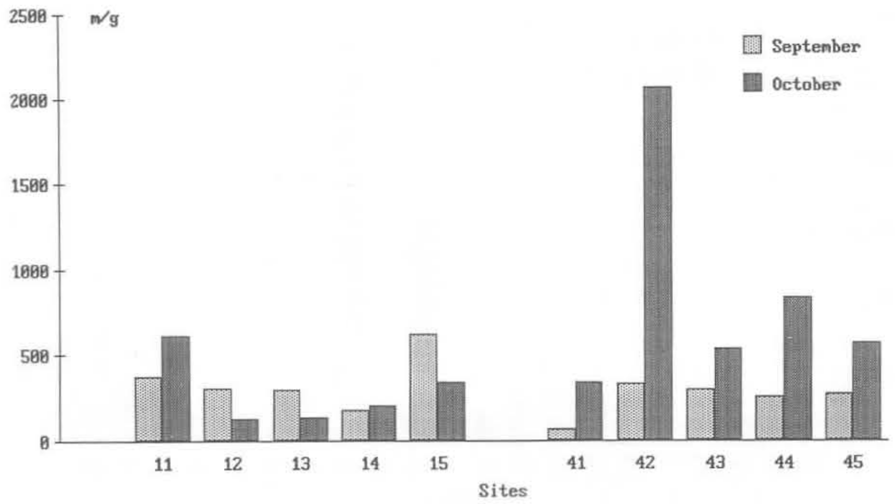


Fig. 6. Amounts of living mycelium in the zones I and IV in September and October 1987.

Chemical soil analyses

Total nitrogen and total sulphur content differed significantly between the zones ($p<0.001$), both being higher in the city centre than in the outermost zones (Table 1). pH was a little higher in the centre, too ($p<0.05$), whereas in the case of mineral nitrogen no significant zonal differences emerged (Fig. 7).

Table 1. Chemical analyses of the humus at the sites. Means of 5 measurements of pH, 4 of total nitrogen and 2 of total sulphur. Sites and zones as in Fig. 1.

Zone	I			II			
Site	pH	N %	S ppm	Site	pH	N %	S ppm
11	4.04	1.22	1290	21	4.01	1.27	2090
12	3.85	1.04	1730	22	3.90	1.07	1510
13	3.93	1.12	1840	23	3.97	1.07	1810
14	3.93	0.95	1550	24	3.98	1.21	2020
15	3.92	0.79	1480	25	3.83	1.07	2000

Zone	III			IV			
Site	pH	N %	S ppm	Site	pH	N %	S ppm
31	4.03	1.63	2610	41	3.98	1.16	2140
32	3.96	1.35	2300	42	3.96	1.31	2210
33	4.08	1.45	2390	43	4.00	1.42	2460
34	4.03	1.56	2230	44	4.12	1.54	2790
35	4.05	1.37	2300	45	4.05	1.46	2450

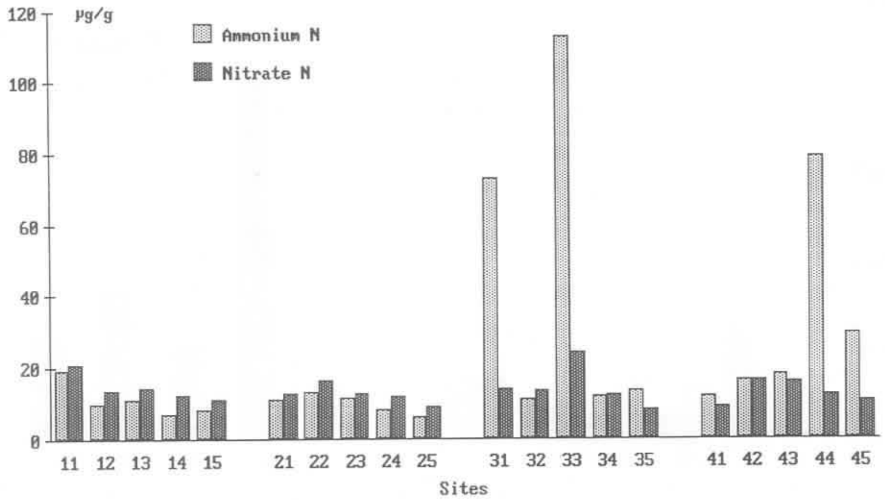


Fig. 7. Ammonium and nitrate nitrogen content of the humus layer in 1987. Means of 3 masurements.

The zones were in any case not uniform in the chemical parameters of their sites, for there were sigificant differences between sites in zones I and II in pH ($p<0.05$), total N concentration ($p<0.001$), ammonium nitrogen ($p<0.01$) and nitrate nitrogen ($p<0.05$). No paired comparisons have yet been made but it seems that site 11 is naturally more eutrophic than the others in zone I or II, as reflected in mineral nitrogen (Fig. 7) and pH (Table 1), for example.

A significant difference between sites was observed in zone III in the case of ammonium nitrogen ($p<0.001$), due to two sites which were very rich in ammonium, 31 and 33 (Fig. 7). Significant differences in soil moisture ($p<0.001$) and total nitrogen ($p<0.05$) were found in zone IV, site 45 being drier than any other site on every sampling occasion and poorer in nitrogen than other sites in zone IV.

This kind of variety in the results may give some interesting information about what is happening in soil under stress from air pollution, and some ideas may be obtained from the correlations between the parameters measured (Table 2). These suggest that high pH, ammonium nitrogen, total nitrogen and total sulphur might tend to reduce sporophore production and the soil respiration rate, while high total sulphur also seems to reduce dehydrogenase activity. It is diffucult in this way to say which of the changes are most detrimental to the microorganisms, however, or what interactions exist between these stress factors. We still have no determinations of heavy metals or exchangeable cations, for example.

Biomass and number of sporophores/m^2, humus respiration rate and dehydrogenase activitiy were correlated with each other, showing that all of

these parameters are suitable indicators of changes in forest ecosystems due to the air pollution. Respiration and dehydrogenase activity in particular are descriptive of the decomposition of organic material and thus are directly linked with the nutrient cycle in the ecosystem.

Table 2. Significance of the coefficients of correlation between certain parameters measured. Sporophores are from 1988. The others are calculated from the means of all measurements at each site in 1987. Positive correlations: *** = $p<0.001$; ** = $p<0.01$; * = $p<0.05$; negative correlations: --- = $p<0.001$; -- = $p<0.01$; - = $p<0.05$.

number of mycorrhizal species											
biomass of sporophores	***										
number of sporophores	***	***									
proportions of healthy mycorrhizae											
humus respiration	***	***	***								
dehydrogenase activity	*	*	*		***						
living mycelium											
pH	--	--	---		-						
ammonium nitrogen	-	-	-		-			**			
nitrate nitrogen						***			*		
total nitrogen	---	--	--		--			***	**		
total sulphur	---	---	--		---	-		**	*		***

Literature

BLACK, C. A., EVANS, D. D., WHITE, J. L., ENSMINGER, L. E., CLARK, F. E. & DINAUER, R. C. 1965. Methods of soil Analysis. Part 2. Chemical and Microbiological Properties. Agronomy 9. Am. Soc. Agr. Inc. Publ.

COLEMAN, D. C. 1973. Compartmental analysis of "total soil respiration": An exploratory study. Oikos 24: 361–366.

KUBIN, E. 1978. Kasvimateriaalin typpipitoisuuden määrittämisestä (Abstract: The determination of the organic nitrogen in plant material). Oulun yliopiston kasvitieteen laitoksen monisteita no. 7 (Mimeogr.).

LEHTO, T. 1984. Kalkituksen vaikutus männyn mykoritsoihin. (Summary: The effect of liming on the mycorrhizae of Scots pine.) Folia For. 609: 1–16.

MALINEN, P. 1983. Dehydrogenaasiaktiivisuuden määrittäminen humuksesta. – Mikrobien kokonaisaktiivisuuden mittaus. Oulun yliopiston kasvitieteen laitos. 13 pp. (Mimeorgr., in Finnish)

MARKKOLA, A. M. & OHTONEN, R. 1988. Mycorrhizal fungi and biological activity of humus layer in polluted pine forests in the surroundings of Oulu. Karstenia 28: 45–47.

OHTONEN, R., MARKKOLA, A. M. & TORVELA, H. 1989. Total sulfur content in the humus layer of urban polluted forest soils. Water Air Soil Poll. 44: 135–141.

OLSON, F. C. W. 1950. Quantitative estimates of filamentous algae. Trans. Am. Microsc. Soc. 59: 272–279.

SÖDERSTRÖM, B. E. 1977. Vital staining of fungi in pure cultures and in soil with fluorenscein diacetate. Soil Biol. Biochem. 9: 59–63.

THALMAN, A. VON, 1968. Zur Methodik der Bestimmung der Dehydrogenaseaktivität im Boden mittels Triphenyltetrazoliumchlorid (TTC). Landw. Forsch. 21: 249–258.

VUONONVIRTA, P., LAITINEN, L. & MIKKONEN, K. 1984. Ilman laatu Oulussa vuosina 1979–1983. Ymäristö ja Terveys 15: 156–161. (in Finnish).